

#### **AMENDMENTS TO THE SPECIFICATION**

Please replace the paragraph on page 1, lines 14-22 with the following amended paragraph:

-- The most well known bacteriocin is nisin, which is the only bacteriocin currently authorised as a food additive. Nisin is produced by fermentation of the dairy starter culture bacterium *Lactococcus lactis* subsp. *lactis*, and is sold as the commercial extract **Nisaplin** **NISAPLIN®** Natural Antimicrobial (Danisco). Nisin has an unusually broad antimicrobial spectrum for a bacteriocin, being active against most Gram-positive bacteria (e.g. species of *Bacillus*, *Clostridium*, *Listeria*, lactic acid bacteria). It is not normally effective against Gram-negative bacteria, yeasts or moulds. Nisin is allowed as a food preservative worldwide but its levels of use and approved food applications are strictly regulated, varying from country to country.--

Please replace the paragraph beginning on page 6, line 22 and ending on page 7, line 6 with the following amended paragraph:

-- Nisin is reportedly a collective name representing several closely related substances which have been designated nisin compounds A, B, C, D and E (De Vuyst, L. and Vandamme, E. J. 1994. Nisin, a lantibiotic produced by *Lactococcus lactis* subsp. *lactis*: properties, biosynthesis, fermentation and applications. In: Bacteriocins of lactic acid bacteria. Microbiology, Genetics and Applications. Eds.: De Vuyst and Vandamme. Blackie Academic and Professional, London). The structure and properties of nisin are also discussed in the article by E. Lipinska, entitled "Nisin and Its Applications", The 25th Proceedings of the Easter School in Agriculture Science at the University of Nottingham, 1976, pp. 103-130 (1977), which article is hereby incorporated by reference. In 1969 the FAO/WHO Joint Expert Committee on Food Additives set specifications for the purity and identity of nisin (FAO/WHO Joint Expert Committee on Food Additives. 1969. Specifications for identity and purity of some antibiotics. 12.sup.th Report. WHO Technical Report Series No. 430). This committee recognised nisin as a safe and legal preservative based on extensive toxicological testing. Nisin has the food additive number E234 and is classed as GRAS (Generally Recognised As Safe) (Food and Drug Administration. 1988. Nisin preparation: Affirmation of GRAS status as a direct human ingredient. Federal Regulations 53: 11247). The international activity unit (IU hereinafter) was

defined as 0.001 mg of an international nisin reference preparation. **Nisaplin NISAPLIN®** Natural Antimicrobial is the brand name for a nisin concentrate containing 1 million IU per g, which is commercially available from Danisco.--

Please replace the paragraph on page 7, lines 8-24 with the following amended paragraph:

-- Nisin is an acknowledged and accepted food preservative with a long history of safe, effective food use. There have been several reviews of nisin, e.g. Hurst 1981; 1983; Delves-Broughton, 1990; De Vuyst and Vandamme, 1994; Thomas et al. 2000; Thomas & Delves-Broughton, 2001). Nisin was discovered over 50 years ago and the first commercial preparation, made in 1953, was **Nisaplin NISAPLIN®**. Nisin has several characteristics that make it particularly suitable as a food preservative. It has undergone extensive toxicological testing to demonstrate its safety. It is heat-stable, acid-stable and effective against a broad spectrum of Gram-positive bacteria. It is not normally effective against Gram-negative bacteria, yeasts or moulds but activity against Gram-negative bacteria and yeasts has been reported in the presence of chelating agents (PCT/US 8902625[[-]], WO 89/12399). Nisin is an effective preservative in pasteurised and heat-treated foods (e.g. processed cheese, cheese, pasteurised milks, dairy desserts, cream, mascarpone and other dairy products, puddings such as semolina, tapioca etc., pasteurised liquid egg, pasteurised potato products, soy products, crumpets, pikelets, flapjacks, processed meat products, beverages, soups, sauces, ready to eat meals, canned foods, vegetable drinks) and low acid foods such as salad dressings, sauces, mayonnaise, beer, wine and other beverages.--

Please replace the paragraph on page 33, lines 5-9 with the following amended paragraph:

-- Materials: GUARDIAN™ Rosemary Extract 09 (Danisco) (GRE09). This is a water dispersible deodorised rosemary extract containing 4% phenolic diterpenes and <1% essential oils, extracted from rosemary leaves, combined with the carriers polyoxyethylene sorbitan monooleate (Tween 80) and propylene glycol. A commercial extract of nisin at potency of 1.times.10.sup.6 IU/g: **Nisaplin NISAPLIN®** Natural Antimicrobial (Danisco).--

Please replace the paragraph on page 35, line 6 with the following amended paragraph:

-- Test compounds: GRE09 at 0.1%, 0.5%, Nisaplin NISAPLIN® (Danisco).--

Please replace Table 2 beginning on page 36, line 28 with the following amended Table 2:

Test Conditions	Days until growth reached $10^6$ CFU/ml
Control	6
Nisin at 100 IU/ml	13
Nisin at 250 IU/ml	27
Rosemary extract GRE09 at 0.1%	10
Rosemary extract GRE09 at 0.5%	20
Nisin (100 IU/ml) + GRE09 at 0.1%	34
<u>Nisaplin NISAPLIN®</u> (100 IU/ml) + GRE09 at 0.5%	> 43
<u>Nisaplin NISAPLIN®</u> (250 IU/ml) + GRE09 at 0.1%	> 43
<u>Nisaplin NISAPLIN®</u> (250 IU/ml) + GRE09 at 0.5%	> 43

Please replace the paragraph beginning on page 37, line 1 with the following amended paragraph:

--During the test period (a) Nisaplin NISAPLIN® (100 IU/ml) +GRE09 at 0.5%, (b) Nisaplin® (250 IU/ml)+GRE09 at 0.1%, and (c) Nisaplin NISAPLIN® (250 IU/ml)+GRE09 at 0.5% did not give any total aerobic viable counts above 100 cfu/g.--

Please replace Table 3 beginning on page 38, line 4 with the following amended Table 3:

Test Conditions	Days until growth reached $10^6$ CFU/ml
Control	6
Nisin at 25 IU/ml	13
Rosemary extract GRE09 at 300 ppm	10
Rosemary extract GRE09 at 600 ppm	13
Nisin (25 IU/ml) + GRE09 at 300 ppm	>70
<u>Nisaplin NISAPLIN®</u> (25 IU/ml) + GRE09 at 600 ppm	>70

Please replace Table 4 beginning on page 38, line 25 with the following amended Table 4:

Test Conditions	Days until growth observed (gas production)
Control	2
Nisin at 25 IU/ml	2

Nisin at 50 IU/ml	2
Nisin at 100 IU/ml	7
Rosemary extract GRE09 at 300 ppm	2
Rosemary extract GRE09 at 600 ppm	2
Nisin (25 IU/ml) + GRE09 at 300 ppm	3
Nisin (50 IU/ml) + GRE09 at 300 ppm	>27
Nisin (100 IU/ml) + GRE09 at 300 ppm	>27
<b>Nisaplin NISAPLIN® (25 IU/ml) + GRE09 at 600 ppm</b>	10
<b>Nisaplin NISAPLIN® (100 IU/ml) + GRE09 at 600 ppm</b>	>27

Please replace Table 5 beginning on page 42, line 8 with the following amended Table 5:

Test	Nisin Content	Phenolic diterpene content	Days until growth reached 10 <sup>6</sup> CFU/g	
			<i>L. monocytogenes</i> at 8°C	<i>B. cereus</i> at 15°C
Control	0		3	2
RE28 at 75 ppm	0 IU/g	21 ppm	5	2
<b>Nisaplin NISAPLIN® at 100 mg/kg</b>	100 IU/g	0 ppm	6	3
<b>Nisaplin NISAPLIN® at 250 mg/kg</b>	250 IU/g	0 ppm	16	6
Nisin/Rosemary blend A	100 IU/g	8.4 pm	15	>26
Nisin/Rosemary blend B	250 IU/g	21 ppm	52	>26

Please replace Table 6 beginning on page 42, line 28 with the following amended Table 6:

Test	Nisin	Phenolic diterpene	Days until 10 <sup>6</sup> CFU/g
Control	0	0	4
RE28 at 60 ppm	0 IU/g	16.8 ppm	5
<b>Nisaplin NISAPLIN® at 100 mg/kg</b>	100 IU/g	0 ppm	11
Nisin/Rosemary blend A	100 IU/g	8.4 pm	>76